IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application No.	: 10/626,943	Confirmation No.:	3231
Applicant	: Rubino		
Filed	: July 25, 2003		
TC/A.U.	: 1614		
Examiner	: G. Polansky		
Customer No.	: 38199		
Title	: Parenteral Formulations		

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Commissioner for Patents
P.O. Box 1450
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DECLARATION UNDER 37 CFR §1.132

Sir:

I, Joseph T. Rubino, residing at 4 Sunrise Way, Towaco, New Jersey, 07082, a citizen of United States of America, do declare and state that:

1. I am one of the named joint inventors of the subject matter claimed in the above-identified patent application.

2. I received a PhD in Pharmaceutics from the University of Arizona in 1984. The subject of my doctoral dissertation was the solubilization of drugs using water miscible cosolvents. I spent 7 years as a faculty member at the University of North Carolina at Chapel Hill, School of Pharmacy where I taught graduate and undergraduate courses in pharmaceutics, including an elective course in parenteral products. At the University of North Carolina, I continued to conduct research in drug solubilization and was awarded a contract from the National Cancer Institute to

perform preformulation and formulation studies on experimental anticancer drugs that had significant challenges associated with their formulation as drug products.

In 1991, I joined The DuPont Merck Pharmaceutical Company, where I was employed for 6 years as a Senior and later Principal Research Scientist in parenteral product development.

I joined Wyeth Research in 1997 as a Section Head in Liquid and Parenteral product and process development. My tenure at Wyeth lasted from 1997 to 2010, during which time I occupied positions of increasing responsibility, culminating in Principal Research Scientist IV, Chemical and Pharmaceutical Development. During that time, I was involved in several projects and programs, including the development of CCI-779 and other rapamycin analogs for the treatment of various cancers. I developed, and supervised the development of, a number of CCI-779 formulations, including oral and parenteral dosage forms, to support phase 1 human clinical trials through word-wide marketed product registration. In doing so, I successfully developed formulations and processes for CCI-779 that overcame the difficult and challenging formulation problems associated with this molecule, including those arising from its chemical instability and poor solubility in water.

Attached to this Declaration (Exhibit A) is my Curriculum Vitae which includes a list of my presentations, publications and patent applications.

3. This Declaration is submitted in the above-identified application to provide evidence rebutting the Examiner's obviousness rejection under 35 USC §103(a) in the Office Action dated June 21, 2010 over the combination of US Patent No. 5,362,718 (Skotnicki), US Patent No. 5,516,770 (Waranis), and UK Patent Application Publication No. 2,327,611 (Haeberlin).

4. All data presented in this Declaration was generated in the United States of America by others employed by the assignee and its predecessor under my supervision and during my employment with the assignee and/or its predecessor.

5. Although CCI-779 and rapamycin are macrocyclic molecules having a similar backbone, it was found that their differences in properties made application of various compositions/formulations attempted for rapamycin inapplicable to the formulation of CCI-779.

(a) CCI-779 was predicted to have a higher aqueous solubility in water, but was found to have a lower aqueous solubility than rapamycin. This resulted in a more difficult challenge to formulate CCI-779 at the concentrations required for delivery to a patient.

A significant difference between CCI-779 and rapamycin is their solubilities. Through experiments conducted to date, it was found that CCI-779 is less soluble in aqueous solutions than rapamycin. However, the challenges related to the poor solubility of CCI-779 became more significant as the concentration increased, *i.e.*, when larger doses were utilized. See, page 2, point 4 in the attached Declaration which was filed during opposition proceedings in the corresponding European Patent No. 1 553 940, for a more detailed discussion regarding CCI-779 solubility.

Therefore, a greater degree of solubility increase was required for CCI-779 compared to rapamycin on the basis of higher dose and lower aqueous solubility. This property, therefore, presented problems in formulating CCI-779 in a parenteral composition, particularly for intravenous administration.

(b) Some of the same approaches to CCI-779 formulations were attempted as were taken for rapamycin formulations. In searching for techniques to improve solubility of CCI-779 in parenteral compositions, the structural characteristics of CCI-779 were considered and experiments were performed. In summary, it was determined that the following **would not** sufficiently increase CCI-779 solubility for a successful CCI-779 parenteral composition:

- the formation of CCI-779 salts;
- adjusting the pH of CCI-779 compositions;
- the formation of CCI-779 inclusion complexes using cyclodextrin derivatives; and
- generating CCI-779 emulsions.

6. Contrary to the Examiner's assertion, the compositions in Example 3 of US Patent Publication No. 2007/0142422 (Rubino) and the CCI-779 compositions of the pending claims are substantially identical. Although not specifically recited, the compositions discussed in Example 3 of Rubino contain the components noted in Table A and contain less than 0.5% w/v of impurities.

Table A

Component	Amount(s)
CCI-779	25 mg/mL
d,l-α-tocopherol	0.2% w/v 0.5% w/v 1% w/v
anhydrous citric acid	0.0025% w/v
dehydrated ethanol	39.5% w/v
propylene glycol	q.s.

The compositions shown in Table A lack polysorbate 80 and polyethylene glycol 400, which are components in the compositions of the pending claims. However, neither polysorbate 80 nor polyethylene glycol 400 comes into contact with CCI-779 until dilution of the CCI-779 concentrate, *i.e.*, immediately prior to administration to the patient. Therefore, polysorbate 80 and polyethylene

glycol 400 cannot and do not contribute to the stability of the CCI-779 concentrate element of the composition.

7. The CCI-779 compositions prepared and studied contained both non-oxidative impurities, referred to as total related compounds (TRC), and oxidative impurities. Both TRC and oxidative impurities are present in CCI-779 samples and in the CCI-779 parenteral compositions immediately upon preparation. Oxidative impurities may also be generated after storing CCI-779 compositions.

A number of factors contributed to the formation of oxidative impurities. It was found that formation of oxidative impurities was controlled by the inclusion of d,l-α-tocopherol in CCI-779 compositions. As evidence, example 2 of the present application discusses that a composition containing CCI-779 (25 mg, 2.5% w/v), dehydrated ethanol (0.395 g, 39.5% w/v), anhydrous citric acid (0.025 mg, 0.0025% w/v), d,l-α-tocopherol (0.75 mg, 0.075% w/v), and propylene glycol (q.s. 1.0 mL) has good stability after 24 months in storage at 2-8 °C and room temperature. It was also observed that no significant degradation occurred after 24 months at 5 °C.

These data reinforce the necessity to include d,l-α-tocopherol in the CCI-779 parenteral formulations, regardless of the amount of initial oxidative impurities.

8. In an effort to further demonstrate the importance of including d,l-α-tocopherol in CCI-779 compositions, particularly at an amount of 0.01 to 0.1% w/v, experiments were performed in which CCI-779 compositions were stored in the absence and presence of d,l-α-tocopherol. In these experiments, compositions containing 0.0025% w/v anhydrous citric acid and the following amounts of CCI-779 and d,l-α-tocopherol were prepared. The amount of TRC in the samples was then measured. See, Table B.

Table B

Time (months)	Temperature (°C)	TRC	CCI-779 Concentration (mg/mL)
0% w/v d,l-α-tocopherol			
0	40	2.45	25.2
1		6.96	22.8
2		14.78	21.5
3	RT	5.24	25.0
8		7.74	23.4
8	4	2.73	24.9
0.075% w/v d,l-α-tocopherol			
0	40	2.41	25.5
1		4.18	24.8
2		5.36	24.6
3		5.07	24.6
3	RT	2.54	25.1
8		2.25	24.8
8	4	2.31	25.3

These data illustrate that CCI-779 substantially degrades in samples lacking d,l-α-tocopherol as compared to samples containing d,l-α-tocopherol. In view thereof, the inclusion of about 0.01 to about 0.1% w/v of d,l-α-tocopherol in the claimed CCI-779 compositions is a critical stabilizing factor.

9. The importance of the claimed d,l-α-tocopherol range of 0.01 to 0.1% w/v in the CCI-779 compositions is further supported by the following data. Specifically, experiments were performed to measure the effect of varying concentrations of d,l-α-tocopherol in CCI-779 compositions. Specifically, efforts were made to determine the minimum concentrations of d,l-α-tocopherol which were required to stabilize CCI-779 compositions and to determine if sufficient residual antioxidant stabilizes CCI-779 compositions over the shelf-life the composition.

Each composition contained 0.0025% w/v anhydrous citric acid, about 5 or about 25 mg/mL of CCI-779, and amounts of d,l-α-tocopherol varying in concentrations from 0 to 0.075% w/v. See, the following Table C.

Table C

Initial			After 1 month at about 40°C		
d,l-α-tocopherol (%w/v)	**Potency of CCI-779 (mg/mL)**	**TRC (%)**	**d,l-α-tocopherol (%w/v)**	**Potency of CCI-779 (mg/mL)**	**TRC (%)**
0	4.98	1.06	0	4.27	13.5
0.019	4.96	1.13	0.015	4.72	5.18
0.038	5.00	0.99	0.031	4.71	5.34
0.056	5.06	1.07	0.047	4.72	6.05
0.070	4.84	1.12	0.059	4.58	5.07
0	25.1	0.97	0	15.4	35.6
0.020	25.4	0.97	0.012	23.9	4.84
0.040	25.2	0.96	0.028	23.8	4.71
0.056	NT	0.95	0.043	23.8	5.22
0.077	25.4	0.95	0.061	24.0	4.96

These data indicate a significant loss of CCI-779 potency after 1 month at 40°C in CCI-779 compositions lacking d,l-α-tocopherol. However, all CCI-779 compositions containing d,l-α-tocopherol significantly reduced degradation of CCI-779. This is evidenced by the potency of CCI-779 and the amount of total related compounds present after 1 month.

10. In addition to poor water solubility, it was found that CCI-779 degraded via several chemical routes. These degradation pathways are summarized in a Declaration which was filed during opposition proceedings in the corresponding European Patent No. 1 553 940 (Exhibit A). This Declaration discusses routes of degradation which were unknown to occur with rapamycin at the time of filing this application. During these studies, it was also found that the CCI-779 compositions contained unacceptable levels of metals as described on pages 5-6, point 7.2 of the Declaration which was filed during opposition proceedings in the corresponding

European Patent No. 1 553 940 (Exhibit A)[1]. One particular metal impurity was found to be zinc.

Experiments were conducted in an effort to chelate the zinc contaminant present in the CCI-779 compositions, which was likely catalyzing CCI-779 degradation. Citric acid was selected over other chelating agents, such as EDTA, EDTA salts, and glycine, because of its greater solubility in the selected cosolvent mixture and its inhibitory effect on metal-catalyzed degradation of CCI-779.

In order to confirm its chelating ability, experiments were performed in which a zinc source, *i.e.*, $ZnCl_2$, and citric acid was added to CCI-779 compositions and maintained at 1 week at 40 °C. The CCI-779 composition contained 50% w/v of ethanol and 0.075% w/v of d,l-α-tocopherol. See Table D which provides the amounts of $ZnCl_2$ and citric acid added to the CCI-779 compositions.

Table D

Zn (ppm)	**citric acid (% w/v)** 0.001	0.0025	0.005
	Amount of Metal-Related CCI-779 Degradant (% area)		
1	0.047	0.018	1
5	0.614	0.204	0.125
10	0.860	0.296	0.172
15	0.976	0.304	0.178
20	2.794	0.334	0.191

These data confirmed that compositions containing 0.001% w/v to 0.005% w/v of citric acid prevented **significant** degradation of CCI-779 in the presence of up to about 20 ppm Zn^{2+}.

[1] Due to repeated copying, Figure 6 on page 6 of the European Declaration is very faded. For clarification, Attached as Appendix B is a replacement drawing for Figure 6 on page 6 of the European Declaration.

11. The amount of citric acid in the claimed CCI-779 compositions is critical. Haeberlin discusses that at least 0.05% w/v of an acid, such as malonic or citric acid, must be added to their compositions. This lower acid limit of Haeberlin far exceeds the maximum amount of citric acid permitted in CCI-779 compositions claimed in the present application. Specifically, compositions containing greater than 0.005% w/v of citric acid degraded faster than compositions lacking citric acid.

As evidence, experiments were conducted in an effort to study the stability of CCI-779 compositions containing varying amounts of citric acid. Four (4) compositions were prepared (runs 1-4) and contained 25 mg/mL of CCI-779, 0.075% w/v of d,l-α-tocopherol, 0% or 0.01% of citric acid, and 30 or 50% w/v of dehydrated ethanol as noted in Table E. The compositions were added to type I glass vials and the final volume adjusted using propylene glycol. The vials were than maintained at 40°C for 1 month and the concentration of total related compounds then measured.

Table E

Run	**Ethanol (% w/v)**	**Citric Acid (% w/v)**	**Initial**	**1 month at 40°C**
			TRC (%)	
1	50	0.01	2.45	13.73
2	50	0	2.18	5.26
3	30	0.01	2.26	23.18
4	30	0	2.19	6.06

These data confirmed that CCI-779 compositions containing 0.01% w/v of citric acid (Runs 1 and 3) were less stable than the same CCI-779 compositions lacking citric acid (Runs 2 and 4), *i.e.*, 0.01% w/v of citric acid was not effective at stabilizing the CCI-779 compositions. One of skill in the art would also be able to extrapolate these data in Table E and conclude that similar CCI-779 compositions containing 0.05% of citric acid **would not** be stable and would result in CCI-779 degradation having very high levels of TRC.

Further experiments were conducted to determine the stability of CCI-779 compositions containing less than 0.01% w/v of citric acid. Four (4) compositions (runs 5-8) were prepared and contained 25 mg/mL of CCI-779, 0.075% w/v of d,l-α-tocopherol, 0.0025% w/v of citric acid, about 39% w/v of dehydrate ethanol, and about 50% w/v of propylene glycol. Four (4) compositions (runs 9-12) were prepared and contained 25 mg/mL of CCI-779, 0.075% w/v of d,l-α-tocopherol, 0.005% w/v of citric acid, about 39% w/v of dehydrate ethanol, and about 50% w/v of propylene glycol. The amount of total related compounds was immediately calculated for Runs 5 and 9. The compositions of Runs 6-8 and 10-12 were retained at the temperature and relative humidity for the periods of time noted in Table F and the total related compounds then calculated.

Table F

Run	Time	Temperature (°C)	Relative Humidity (%)	TRC (%)	CCI-779 Concentration (mg/mL)
0.0025% w/v citric acid					
5	0	n/a	n/a	0.90	25.4
6	1 month	40	75	1.60	25.2
7	3 months	25	60	1.17	24.7
	6 months			1.64	24.4
8	6 months	5	n/a	1.05	24.5
0.005% w/v citric acid					
9	0	n/a	n/a	1.05	24.9
10	1 month	40	75	4.09	23.0
11	6 weeks	25	60	1.41	24.6
	6 months			3.32	23.5
	9 months			3.29	23.6
	12 months			5.15	23.3
12	108 days	5	n/a	1.17	24.9
	6 months			1.24	24.4
	9 months			1.25	24.4
	12 months			1.21	24.6

These data illustrate 0.0025 to 0.005% of citric acid were highly effective in controlling escalation of TRC and maintaining potency of the CCI-779 compositions.

In fact, the data from Tables E and F are comparable to further evidence the effectiveness of about 0.001 to 0.005% w/v range of citric acid in the claimed compositions. Specifically, amounts of citric acid above 0.005% w/v in CCI-779 compositions did not result in stable compositions.

12. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date: 15-MAR-2011 By: Joseph T. Rubino

Joseph T. Rubino

EXHIBIT A

DECLARATION

European Patent EP 1 553 940 B1 of Wyeth LLC

I, Joseph T. Rubino, residing at 4 Sunrise Way, Towaco, New Jersey, 07082, a citizen of United States of America, do declare and state that:

1. I received a PhD in Pharmaceutics from the University of Arizona in 1984. The subject of my doctoral dissertation was the solubilization of drugs using water miscible cosolvents. I spent 7 years as a faculty member at the University of North Carolina at Chapel Hill, School of Pharmacy where I taught graduate and undergraduate courses in pharmaceutics, including an elective course in parenteral products. At the University of North Carolina I continued to conduct research in drug solubilization and was awarded a contract from the National Cancer Institute to perform preformulation and formulation studies on experimental anticancer drugs that had significant challenges associated with their formulation as drug products.

In 1991 I joined The DuPont Merck Pharmaceutical Company, where I was employed for six years as a Senior and later Principal Research Scientist in parenteral product development.

I joined Wyeth Research in 1997 as a Section Head in Liquid and Parenteral product and process development. My tenure at Wyeth lasted from 1997 to 2010, during which time I occupied positions of increasing responsibility, culminating in Principal Research Scientist IV, Chemical and Pharmaceutical Development. During that time I was involved in several projects and programmes, including the development of CCI-779 and other rapamycin analogues for the treatment of various cancers. I developed, and supervised the development of, a number of CCI-779 formulations, including oral and parenteral dosage forms, to support phase 1 human clinical trials through to world-wide marketed product registration. In so doing I successfully developed formulations and processes for CCI-779 that overcame the difficult and challenging formulation problems associated with this molecule, including those arising from its chemical instability and poor solubility in water.

I have annexed to this declaration my Curriculum Vitae, including a list of my presentations, publications and patent applications.

I am one of the inventors of the subject matter of European Patent EP 1 553 940 B1.

2. At the request of Wyeth's EPO representatives, Mr. Graham Lane and Mr Keith Ruddock, I have reviewed the Opposition Division's Communication of 17 September 2010 in relation to EP 1 553 940 B1, wherein it is noted that: "*At present, the Opposition Division has no problem to accept that the technical effect of enhancement of physicochemical stability of CCI-779 in concentrate and parenteral formulations is credibly shown in the contested patent ... it is however true that the experimental evidence provided in the contested patent is very limited.*"

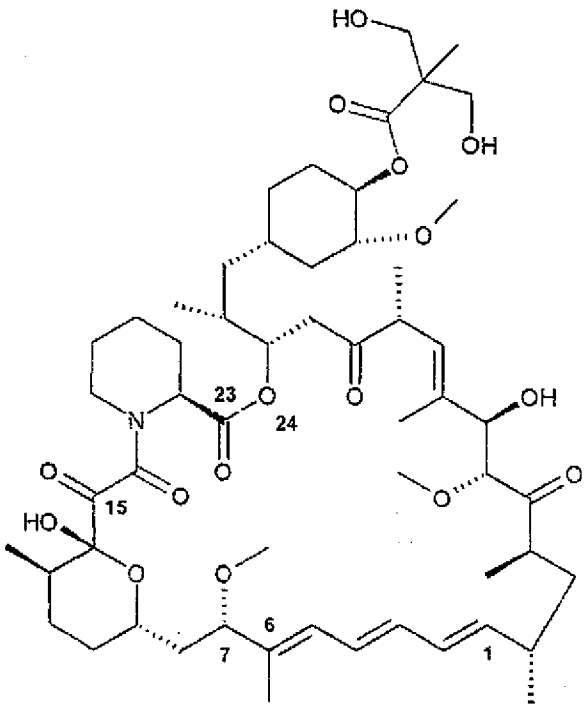
(page 7, towards the end of the first full paragraph).

3. Consequently, I have reviewed the data available at Wyeth concerning the stability of CCI-779 (also known as temsirolimus) cosolvent concentrate and parenteral formulations. These data result from a research program to this end undertaken by Wyeth over many years.

4. CCI-779 is a macrocyclic ester, the structure of which is set out overpage, wherein positions of particular relevance to potential degradation have been numbered, for ease of reference.

The aqueous solubility of CCI-779 is less than 1.1 mcg/mL. Since there are no ionizable functional groups in the molecule, the solubility is independent of pH. The partition coefficient of CCI-779 in an octanol/water system was found to be high (Log PC > 4.1) indicating a high degree of lipophilicity. The solubility of CCI-779 in several water miscible cosolvents has been determined and includes dehydrated alcohol (>500 mg/mL), propylene glycol (33 mg/mL) and polyethylene glycol 400 (27.4 mg/mL). CCI-779 solubility in polysorbate 80 is 16.5 mg/mL.

CCI-779 is much less soluble in oils such as olive oil (0.13 mg/mL) and mixed glycerides of intermediate chain length (e.g. Miglyol 812 solubility is 0.86 mg/mL).



CCI-779

5. As set out below, there are multiple degradation pathways that are potentially significant for CCI-779, some of which are unique to CCI-779 cosolvent concentrate for injection, as claimed in EP 1 553 940 B1.

5.1 Ester Hydrolysis at Position 23-24. As a macrocyclic ester, cleavage of the ester bond at position 23-24 can occur to form seco-CCI-779. This pathway is catalysed primarily by base, but also occurs under acidic conditions or in the presence of metals. Seco-CCI-779 is the primary degradation product observed in admixtures of CCI-779 with aqueous infusion solutions, such as 0.9% sodium chloride injection.

5.2 Substitution at Position 7. This acid catalysed pathway, which is unique to CCI-779 concentrate for injection, is relevant where the parenterally acceptable cosolvent is alcoholic. For example, where the cosolvent is ethanol, substitution of ethyl for methyl at the 7-ether position can occur. The presence of oxidative degradation products in drug substance can also act as a significant catalyst for this substitution. In addition, this substitution is catalysed by metal salts, including the chloride salts of Fe, Al, Ni and Mn.

5.3 Ester Formation and ring rearrangement at Position 15. This pathway, also unique to CCI-779 concentrate for injection, is relevant where the parenterally acceptable cosolvent is alcoholic. Ester formation is catalysed by certain transition metals such as Zn, Ni, Cu and Mn.

5.4 Oxidation of C-1-6 Triene Region. Oxidation of the carbon 1-6 triene region can occur in CCI-779. Numerous degradation products result therefrom and include oxygen addition compounds, epoxides and hydroxides. The presence of oxidative/hydrolytic impurities in drug substance is also autocatalytic for CCI-779.

6. The concentrate for injection that was developed to support phase I clinical trials consisted of a solution of CCI-779 25 mg/mL in dehydrated alcohol with citric acid as a stabilizer. Although this early dosage form provided dosing flexibility for early clinical studies, the flammability of a formula containing nearly 100% alcohol was a significant safety hazard. In addition, some clinical batches demonstrated rapid degradation due to oxidation of CCI-779.

7. Studies were initiated, therefore, to develop a modified concentrate for injection which would be less flammable and, importantly, provide better protection against degradation via the routes discussed in (5) above.

7.1 Oxidative Degradation of CCI-779. A series of CCI-779 stability experiments using citric acid, BHT and d,l-α-tocopherol were conducted. Sixteen concentrates, the precise compositions of which are given in Table 1 and which included control formulae 4 and 10, were prepared at a CCI-779 concentration of 25 mg/mL, filled into type I glass vials and examined at 1 and 2 months after storage at 40°C. The corresponding data are presented in Table 2.

Based on results for strength (i.e. remaining CCI-779), total related compounds (TRC) and observations regarding appearance, acceptable CCI-779 stability was provided by the concentrates of formulae 6, 8, 11, 13, 14 and 16, i.e. concentrates containing BHT, d,l-α-tocopherol and mixtures thereof as antioxidant.

The concentrate of formula 14, containing d,l-α-tocopherol, and set out below for ease of reference, provided the best protection against degradation of CCI-779 .

CCI-779	25 mg/mL
dehydrated alcohol	50% v/v (39.5%w/v)
d,l-α-tocopherol	0.075%w/v
propylene glycol	q.s.

7.1.1 To further verify the effectiveness of d,l-α-tocopherol in preventing oxidation of CCI-779, several ampoules from a previously manufactured batch of CCI-779 cosolvent concentrate (CCI-779 25 mg/mL, citric acid 0.005% w/v in dehydrated alcohol) were opened and the contents removed. Half the volume was spiked with an alcoholic concentrate containing d,l-α-tocopherol while the remaining solution was not. The two solutions were filled into fresh ampoules and their stability examined. The data is summarized in Table 3.

The ampoules that were spiked with d,l-α-tocopherol had enhanced CCI-779 stability at 40°C and room temperature storage compared to the control group with no antioxidant, thereby demonstrating that the addition of the antioxidant d,l-α-tocopherol improves the stability of CCI-779.

7.1.2 In addition, a small batch (1L) of CCI-779 cosolvent concentrate for injection prepared according to formula 14. The cosolvent concentrate was prepared as a 1 mL fill in a 2 mL flint glass vial and the stability of CCI-779 examined over 24 months. The CCI-779 cosolvent concentrate for injection of formula 14 demonstrated excellent stability, with little degradation evident at 5°C for up to 24 months.

7.2 Effect of Metals on Degradation of CCI-779. Table 4 illustrates the effects of various metals on the degradation of CCI-779 in a cosolvent concentrate of formula 14 after 1 week storage at 40°C.

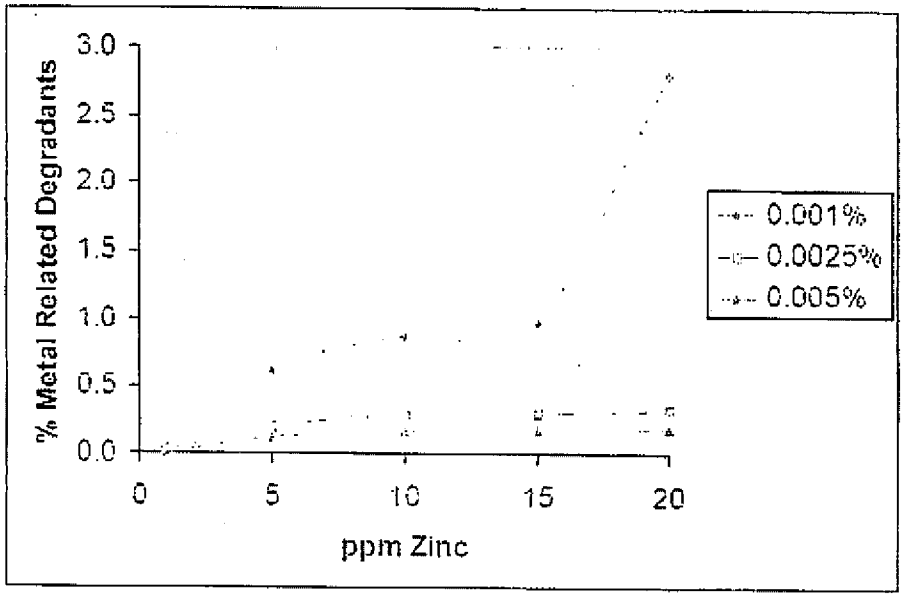
Exposure of the formulated CCI-779 solution to type 316L stainless steel for 24 hours resulted in degradation via the formation of alcohol esters. This is most likely due to extraction of metals by the CCI-779 vehicle since exposure of the vehicle alone to metal, followed by addition of drug, resulted in the characteristic metal degradants. The effects

of individual metals were also examined. Copper and nickel metal each resulted in the formation of alcohol esters. In the case of nickel and copper, the elemental metal forms were pre-equilibrated with propylene glycol prior to formulation of CCI-779.. This resulted in extraction of enough metal to cause the reaction.

7.2.1 In order to further improve the robustness of the CCI-779 concentrate formula against metal catalysis a chelating agent, citric acid anhydrous, was investigated. Concentrations of 0.001, 0.0025 and 0.005% w/v were added to the d,l-α-tocopherol containing formula 14, which was spiked with 1-20 ppm $ZnCl_2$.

Table 5, represented graphically in Figure 1 below, illustrate the effects of citric acid and zinc concentration on the presence of all metal related degradants after solutions were stressed at 40°C for one week.

Figure 1



These data indicate that 0.001% w/v citric acid was able to protect CCI-779 from the action of Zn^{2+} at lower concentrations, and that 0.0025% and 0.005% w/v concentrations of citric acid were able to prevent significant degradation of CCI-779 in the presence of up to 20 ppm Zn^{2+}.

7.2.2 Longer term stability data of CCI-779 concentrates containing 0.005% and 0.0025% anhydrous citric acid are presented in Tables 6 and 7. The 0.0025% concentration resulted in a slightly more stable product under stress conditions.

Accordingly the formula of CCI-779 concentrate that was ultimately developed for commercialisation included 0.0025% anhydrous citric acid to inhibit catalysis by metals and had the formula below.

CCI-779 Concentrate for Injection, 25mg/mL:

Component	Concentration
CCI-779	25 mg/mL
d,l-α-tocopherol	0.075%
Citric acid, anhydrous	0.0025%
Dehydrated alcohol	39.5% w/v/
Propylene glycol	q.s.

8. The data reported herein clearly demonstrate that CCI-779 cosolvent concentrates and parenteral formulations falling within the claims of EP 1 553 940 B1 presently before the Opposition Division exhibit enhanced stability.

Date: 22-Dec-2010 By: Joseph T. Rubino

Joseph T. Rubino

Table 1 **Composition of sixteen CCI-779 Concentrates Containing Citric Acid, BHT and d,l-α-Tocopherol**

Run	% Ethanol [a]	% Citric Acid	% BHT	% d,l-α-Tocopherol
1	50	0.01	0.02	0
2	30	0.01	0	0
3	50	0.01	0	0.075
4	50	0	0	0
5	50	0.01	0.02	0.075
6	30	0	0.02	0
7	50	0.01	0	0
8	50	0	0.02	0
9	30	0.01	0.02	0.075
10	30	0	0	0
11	30	0	0	0.075
12	30	0.01	0.02	0
13	30	0	0.02	0.075
14	50	0	0	0.075
15	30	0.01	0	0.075
16	50	0	0.02	0.075

a: %v/v Dehydrated alcohol in formula. Final volume adjusted using propylene glycol.

Table 2 Stability of the Sixteen Concentrates of Table 1

	Initial			1 month 40°C			2 months 40°C		
Run	Assay	%TRC	Initial Observation	Assay	%TRC	1mo. Observation	Assay	%TRC	2mo. Observation
1	24.4	2.34	CCS	20.4	17.62	CCS	NT	NT	CCS
2	24.0	2.27	CCS	5.30	78.27	Light Yellow	NT	NT	Light Yellow
3	24.6	2.45	CCS	21.0	13.73	CCS	NT	NT	CCS
4	24.6	2.24	CCS	11.6	54.33	Light Yellow	NT	NT	Light Yellow
5	24.3	2.28	CCS	21.7	13.62	CCS	NT	NT	CCS
6	24.3	2.12	CCS	22.9	3.64	CCS	19.2	22.17	CCS
7	24.6	2.32	CCS	13.0	51.93	Light Yellow	NT	NT	Light Yellow
8	24.6	2.15	CCS	23.2	7.71	CCS	20.7	18.38	CCS
9	24.6	2.20	CCS	19.6	21.10	CCS	NT	NT	CCS
10	24.4	2.23	CCS	5.50	76.89	Light Yellow	NT	NT	Light Yellow
11	24.6	2.19	CCS	23.3	6.06	CCS	21.5	14.29	CCS
12	24.6	2.32	CCS	19.1	22.29	CCS	NT	NT	CCS
13	24.4	2.16	CCS	23.2	7.29	CCS	21.2	14.75	CCS
14	24.9	2.18	CCS	24.0	5.26	CCS	22.4	10.71	CCS
15	24.2	2.26	CCS	19.2	23.18	CCS	NT	NT	CCS
16	24.8	2.36	CCS	23.7	7.79	CCS	22.1	15.67	CCS

TRC = Total Related Compounds; CCS = Clear Colourless Solution; NT = Not Tested

Table 3 **Effect of d,l-α-Tocopherol on Stability of CCI-779 Cosolvent Concentrate in Ampoules**

Sample	Time	Assay: mg/mL (% Initial)	%TRC	Appearance/ Description
		Control-Without d,l-α-tocopherol		
40°C	initial	25.2 (100%)	2.45	CCS
	1 month	22.8 (90.4)	6.96	Pale yellow solution
	2 months	21.5 (85.2)	14.78	Pale yellow solution
Room Temp	3 months	25.0 (99.0)	5.24	CCS
	8 months	23.4 (92.6)	7.74	CCS
4°C	8 months	24.9 (98.9)	2.73	CCS
		Sample-With 0.075% d,l-α-tocopherol		
40°C	initial	25.5 (100%)	2.41	CCS
	1 month	24.8 (97.4)	4.18	CCS
	2 months	24.6 (96.2%)	5.36	CCS
	3 months	24.6 (96.2%)	5.07	Very, very pale yellow solution
Room Temp	3 months	25.1 (98.4%)	2.54	CCS
	8 months	24.8 (97.3%)	2.25	CCS
4°C	8 months	25.3 (99.1%)	2.31	CCS

TRC = Total Related Compounds

CCS = Clear Colourless Solution

Table 4 **Effect of Metals on CCI-779 Total Related Compounds (TRC) after Storage at 40°C**

Sample	Storage Time (weeks)	TRC (% area)
Control – no metal contact	4	0.64
T316L Stainless Steel contact 24 hours [a]	4	3.94
Vehicle Control –no metal contact	4	0.50
T316L Vehicle contact 24 hours [b]	4	6.60
Control –no metal contact	1	0.57
Copper [c]	1	2.28
Control –no metal contact	1	0.50
Nickel [d]	1	11.8
Control –no metal contact	1	0.56
Manganese [e]	1	0.24

a. Formulation exposed to metal cylinder for 24 hours.

b. Vehicle exposed to metal cylinder for 24 hours prior to addition of drug.

c. Propylene glycol equilibrated with metal flakes prior to formulation with drug.

d. Propylene glycol equilibrated with metal flakes prior to formulation with drug.

e. Propylene glycol equilibrated with metal flakes prior to formulation with drug.

Table 5 **Effect on CCI-779 Concentrate of Formula 14 of Citric Acid and Zinc Concentration after Storage at 40°C**

ppm Zn	0.001% citric acid	0.0025% citric acid	0.005% citric acid
1	0.047	0.018	0
5	0.614	0.204	0.125
10	0.860	0.296	0.172
15	0.976	0.304	0.178
20	2.794	0.334	0.191

Data are % area of all metal-related degradants.

Table 6 **Stability Data for CCI-779 Concentrate at 25 mg/mL with d,l-α-tocopherol and 0.005% citric acid anhydrous**

Test	Appearance and Description	TRC (%)	Assay (Strength) mg/mL	d,l-α-tocopherol (%)	Moisture (%)
Storage condition					
Time Zero	Clear, colorless solution essentially free from visual particulates	1.05	24.9	99.0	0.30
40°C/75% RH (inverted)					
1 month	No change	4.09	23.9	NT	NT
25°C/60% RH (inverted)					
6 weeks	No change	1.41	24.6		
6months	No change	3.32	23.5		
9 months	No change	3.29	23.6		
12 months	No change	5.15	23.3	82.4	
5°C (inverted)					
108 days	No change	1.17	24.9		
6 months	No change	1.24	24.4		
9 months	No change	1.25	24.4		
12 months	No change	1.21	24.6	96.8	0.10

Table 7 **Stability Data for CCI-779 Concentrate at 25 mg/mL with d,l-α-tocopherol and 0.0025% citric acid anhydrous**

Test	Appearance and Description	TRC (%)	Assay (Strength) mg/mL	Alpha-tocopherol (%)	Moisture (%)
Storage condition					
Time Zero	Clear, colorless solution essentially free from visual particulates	0.90	25.4	103.3	0.27
40°C/75% (inverted)					
1 month	No change	1.60	25.2		
25°C/60%RH (inverted)					
3months	No change	1.17	24.7		
6months	No change	1.64	24.4	93.3	
5°C (inverted)					
6months	No change	1.05	24.5	103.5	0.26

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CURRICULUM VITAE

I. PERSONAL

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II. EDUCATION

University of Arizona
Tucson, AZ
Doctor of Philosophy (Pharmaceutics)

Philadelphia College of Pharmacy & Science
Philadelphia, PA
Bachelor or Science Degree in Pharmacy ("Cum Laude")

III. EMPLOYMENT HISTORY

2009-2010 Principal Research Scientist IV, Chemical and Pharmaceutical Development Wyeth Research

- Successfully developed the formulation and process for Torisel® from Phase 0 through NDA and worldwide marketing approvals
- Directly participated in FDA Quality by Design (QbD) Pilot Program (Torisel®) as the first parenteral product to be approved using QbD principles
- Co-Leader of Global Technical Transfer Team for Torisel®
- Pharmaceutical Sciences representative to Local and Global Project Development Teams
- Supervise 2-6 technical staff (B.S., M.S., Ph.D. Level) and effectively trained numerous scientists on various laboratory techniques and procedures.
- Successfully developed numerous liquid formulations for early development studies, including oral and parenteral products.
- Development experience includes both antibody-based, peptide and small molecular pharmaceutical agents with poor aqueous solubility &/or chemical stability.
- Development of IV disperse systems for enhanced drug uptake into brain

2004-2008- Principal Research Scientist III, Chemical and Pharmaceutical Development Wyeth Research

2001-2004 Principal Research Scientist I,II/Section Head, Chemical and Pharmaceutical Development Wyeth Research

1997-2001 Section Head, Chemical and Pharmaceutical Development Wyeth Research

1995-1997 Principal Research Scientist, The DuPont Merck Pharmaceutical Company, Pharmaceutical Research and Development

1991-1995 Senior Research Scientist, The DuPont Merck Pharmaceutical Co.

1984-1991 Assistant Professor of Pharmaceutics, University of North Carolina School of Pharmacy (Promotion and Tenure approved July, 1990).

1983-1984 Research Associate, University of Arizona, College of Pharmacy.

1979-1983 Teaching Assistant, University of Arizona, College of Pharmacy.

1977-1978 Registered Pharmacist, Tri State Drug Center, 615 Ave. of States, Chester, PA

1973-1977 Pharmacy Intern, Mel's Marple Pharmacy, 2530 West Chester Pk., Broomall PA

IV. ACADEMIC EXPERIENCE

1984-1991 Assistant Professor of Pharmaceutics, University of North Carolina School of Pharmacy (Promotion and Tenure approved July, 1990)

- Coordinate and participate in teaching undergraduate and graduate courses in physical pharmacy.
- Co-Developed elective courses in Sterile Products and Cosmetic Products
- Developed independent research program in physical pharmacy, total contracts and grants awarded > $400K.

V. ACADEMIC AND PROFESSIONAL HONORS

Awards:

February 2009 President's Research and Development Award, Wyeth Pharmaceuticals

December 2006 President's Achieving Excellence Award, Wyeth Pharmaceuticals

October 1991 Best Paper Published in Journal of Parenteral Science and Technology for the Year 1990, Parenteral Drug Association

Fall 1983 Graduate Student Development Grant, University of Arizona

1980 Foundation — Meritorious Performance as Teaching Assistant, University of Arizona

1979-1984 — Graduate Academic Scholarship, University of Arizona

1977 Science — Merck, Sharpe and Dohme Award, Philadelphia College of Pharmacy and

1972-1973 — Ralph H. Pyle Award, Philadelphia College of Pharmacy and Science

Research Support:

National Cancer Institute, "Development of Dosage Forms and Delivery Systems for New Anti-tumor Agents," June 1989. $366,752.

Parenteral Drug Association Foundation for Pharmaceutical Sciences, Inc., "Studies on the Physical and Chemical Stability for Parenteral Emulsions and Emulsifiers," 1988. $15,000.

Junior Faculty Development Award, "An Investigation into the Factors which Determine the Solubilities of Salt Forms of Weak Electrolyte Drugs," 1988. $3,000.

United Way of North Carolina, Inc., "Development of New Dosage Forms for the Treatment of Urinary Bladder Disease, " 1987. $3,700.

University Research Council, "Physical Studies of Lecithins as Emulsifiers," University of North Carolina, 1987. $1,500.

Parenteral Drug Association Foundation for Pharmaceutical Sciences, Inc., "Estimation of Liquid Vehicle Composition from Physicochemical Parameters," 1985. $15,000.

University Research Council, "Interfacial Studies of Phospholipid-Nonionic Surfactant Combinations," University of North Carolina, 1985. $1,500.

Pharmacy Foundation of North Carolina, Inc., "The Influence of Solvent Composition on the Action of Pharmaceutical Buffer Systems," 1984. $1,500.

Parenteral Drug Association Foundation for Pharmaceutical Sciences, Inc., "Formulation of Poorly Soluble Drugs for Parenteral Use," S.H. Yalkowsky, J.T. Rubino, 1983, $10,000.

VI. PRESENTATIONS AND PUBLISHED ABSTRACTS

Rubino, J.T., Blanchard, J. Armstrong, E.A., Yalkowsky, S.H., "Solubilization of Diazepam and Phenytoin by Cosolvents," Abstracts of the 35th National Meeting of APS 13:156 (1983).

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Dugan, J.E., Rubino, J.T., "Phase Inversion Temperature Analysis of Emulsions Composed of Ionic and Nonionic Surfactants", Pharm. Res. 6:S138 (1989).

Rubino, J. Chan, L., Walker, J., Segretario, J., Hussain, M., "Particulate Formation in an Experimental Antitumor Drug Formulation," 30th Annual Higuchi Research Seminar, March 10, 1997.

Rubino, J.T., "Development Strategies for Improving the Bioavailability of Poorly Water Soluble Drugs. Solubility Theory and Modification of Solubility," Abstracts of SE Regional AAPS Meeting, June 22, 1998, p. 15.

Rubino J., Rahman, M., Phelan, L., Crawford, W., Ocampo, N., "In-Use Stability of Temsirolimus Injection and Compatibility with Intravenous Administration Devices," JAPhA, Mar/Apr, 2008, p. 245.

VII. PUBLISHED ARTICLES AND BOOK CHAPTERS

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VIII. Patents

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US20040192900A1, Calicheamicin derivative-carrier conjugates, September 30, 2004.

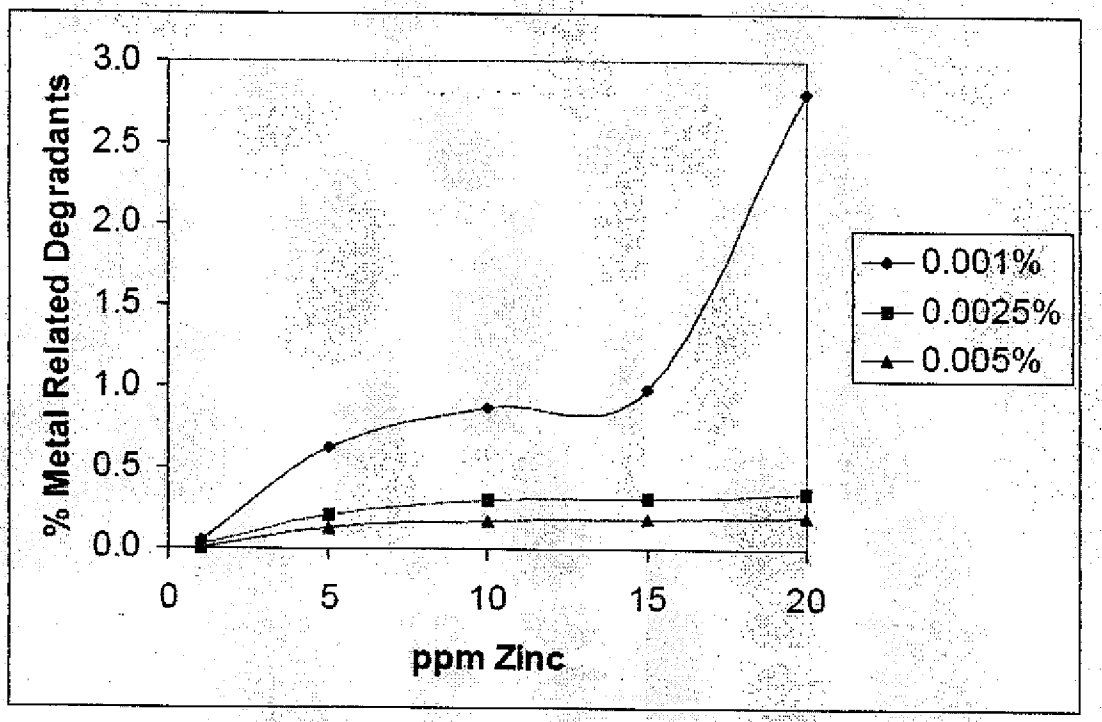
US20050020615A1, CCI-779 Lyophilized Formulations, January 27, 2005.

US20060002942A1, Calicheamicin Conjugates, January 5, 2006.

US20070142422A1, Control of CCI-779 dosage form stability through control of drug substance impurities, June 21, 2007.

US20090105461A1, Calicheamicin Conjugates, April 4, 2009.

EXHIBIT B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2.5
2.0
1.5
1.0
0.5
0.0
0
5
10
15
20
ppm Zinc
0.001%
0.0025%
0.005%